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RESEARCH ARTICLE

Morphological revision of *Simulium* (*Gomphostilbia*) *ogatai* (Rubtsov) in the *S. ceylonicum* species-group (Diptera: Simuliidae) from Japan

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ABSTRACT

Simulium (*Gomphostilbia*) *ogatai* (Rubtsov) from Japan, the only Palaearctic species in the *S. ceylonicum* species-group, is morphologically revised. Its female is described for the first time and its male and pupa are redescribed based on specimens from Gifu, Honshu. It is characterized in the female and male by the base of the radius with a tuft of yellow hairs, in the female by the head slightly wider than the thorax, 5.89 times as wide as the greatest width of the frons, and short sensory vesicle 0.21–0.24 times as long as the third palpal segment; in the male by the number of upper-eye (large) facets in 15 vertical columns and 16 horizontal rows, and hind basitarsus spindle-shaped, enlarged, 3.79 times as long as wide, and 0.83 and 1.00 times as wide as the greatest width of the hind tibia and femur, respectively; and in the pupa by the terminal hooks simple and cone-like, and the cocoon with an anterodorsal projection. Taxonomic notes for this species are given relevant to several related species in the Oriental Region, and its assignment to the *S. ceylonicum* species-group is confirmed.

Keywords: Black fly; taxonomy; biting insects.

INTRODUCTION

The subgenus *Gomphostilbia* Enderlein is the second largest among the 38 subgenera of the genus *Simulium* Latreille. It is represented by 302 species, which are distributed mostly in the Oriental Region and sporadically in the Australasian and Palaearctic Regions (Adler, 2021). Among species of this subgenus, *S. asakoae* Takaoka & Davies and *S. chumpornense* Takaoka & Kuvangkadilok are vectors of an unknown filarial worm and protozoan parasites of the genera *Leucocytozoon* Berestneff and *Trypanosoma* Gruby in Thailand (Fukuda *et al*., 2003; Thaijarern *et al*., 2019; Pramual *et al*., 2020).

In Japan, where 79 species of black flies belonging to five genera are known, the subgenus *Gomphostilbia* is represented by six species in three species-groups: *S. ogatai* (Rubtsov), *S. okinawense* Takaoka, *S. tokarense* Takaoka in the *S. ceylonicum* species-group, *S. omutaense* Ogata & Sasa and *S. yaeyamaense* Takaoka in the *S. batoense* species-group, and *S. shogakii* (Rubtsov) in the *S. varicorne* species-group (Adler, 2021).

Among the three species in the *S. ceylonicum* species-group, *S. ogatai,* distributed in Honshu, Shikoku and Kyushu, is the only species recorded in the Palaearctic Region among the 32 species in this species-group (Adler, 2021). This species was briefly described as a new species, *Eusimulium ogatai*, by Rubtsov (1962) based on the illustrations of the male and pupal characteristics of *Simulium* sp. J-13, which was one of the 14 undescribed Japanese black fly species designated as *S*. sp. J-1 to *S*. sp. J-14 by Bentinck (1955). No type specimen was designated for *S. ogatai* by Rubtsov (1962). However, when Rubtsov applied the name *S. ogatai* for *S*. sp. J-13, the species was known only from one drainage ditch in Kyoto Prefecture (exact location unspecified). So, although a type specimen does not exist, at least the concept of *S. ogatai* is based on material from Kyoto Prefecture. The larval head capsule of this species was illustrated in dorsal and ventral views by Orii *et al*. (1969). This species was placed in the *S. ceylonicum* species-group, redefined by Takaoka (2012), based on the shape of the ventral plate of the male genitalia illustrated by Bentinck (1955), which is narrowed posteriorly when viewed ventrally, and the enlarged male hind basitarsus noted in the key provided by Bentinck (1955).

In the present study, we describe the female of *S. ogatai* for the first time, and redescribe its male and pupa based on specimens collected from Gifu Prefecture, Honshu, Japan.

MATERIALS AND METHODS

Specimens of *S. ogatai* used for morphological observation and description or redescription are one female and one male reared from pupae, with their associated pupal exuviae and cocoons, collected from a stream (branch of Fukazawa River) (35°22'28''N, 137°09'28''E), Gotomaki, Toki City, Gifu Prefecture, Honshu, Japan, 17-VII-2011, by Y. Otsuka. All specimens were fixed in 80% ethanol.

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The methods of collection, description and illustration, and terms for morphological features, follow those of Takaoka (2003) and partially those of Adler *et al*. (2004).

The specimens used are deposited in Research Center for the Pacific Islands, Kagoshima University, Kagoshima, Japan.

RESULTS AND DISCUSSION

***Simulium* (*Gomphostilbia*) *ogatai* (Rubtsov, 1962)**

Female (n=1). Body length 2.0 mm. ***Head***. Slightly wider than thorax. Frons dark brown, densely covered with yellowish white scale-like recumbent short hairs interspersed with few dark longer hairs along each lateral margin; frontal ratio 1.39:1.00:2.65; frons:head ratio 1.00:5.89. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus dark brown, densely covered with yellowish white scale-like hairs interspersed with several dark longer hairs on each side. Labrum 0.59 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres, light to medium brown except scape, pedicel and base of first flagellomere yellow. Maxillary palpus composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.00:1.09:2.50; sensory vesicle (Figure 1A) ellipsoidal, short (0.21–0.24 times length of third segment), with relatively large opening. Maxillary lacinia with 12–14 inner and 15 or 16 outer teeth. Mandible with 27 inner teeth and five outer teeth at some distance from tip. Cibarium (Figure 1B) medially forming sclerotized plate folded forward from posterior margin, with weakly sclerotized mediolongitudinal ridge with dark bifid apex. ***Thorax***. Scutum dark brown to brownish black except anterolateral calli ochreous, shiny when illuminated at certain angles, with three faint dark longitudinal vittae, densely covered with yellow scale-like recumbent short hairs even on median and two submedian longitudinal vittae. Scutellum medium brown, covered with yellowish white short hairs and dark brown long upright hairs along posterior margin. Postnotum dark brown, slightly shiny when illuminated at certain angles, and bare. Pleural membrane ochreous and bare. Katepisternum longer than deep, medium to dark brown, shiny when illuminated at certain angles, moderately covered with fine yellow and brown short hairs. ***Legs***. Foreleg: coxa whitish yellow; trochanter whitish yellow, with small portion somewhat darkened; femur light brown with apical cap medium brown (though extreme tip yellowish); tibia yellowish white except apical three-tenths brownish black; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.12 times as long as its greatest width. Midleg: coxa medium brown except posterolateral surface dark brown; trochanter yellow, with small portion somewhat darkened; femur light brown with apical cap medium brown (though extreme tip yellowish); tibia whitish yellow on basal one-third and light to dark brown on rest; tarsus dark brown to brownish black though basal half of basitarsus yellow. Hind leg: coxa light brown; trochanter yellow; femur medium brown with base yellow and apical cap dark brown (though extreme tip yellowish); tibia (Figure 1C) yellowish white on basal half, then light gray on middle one-fifth and dark brown to brownish black on apical one-third; tarsus dark brown except basal two-thirds (though base light brown) and basal half of second tarsomere white to yellowish white; basitarsus (Figure 1D) narrow, nearly parallel-sided, though slightly narrowed apically, 6.73 times as long as wide, and 0.65 and 0.50 times as wide as greatest widths of tibia and femur, respectively; calcipala (Figure 1D) nearly as long as width at base, and 0.55 times as wide as greatest width of basitarsus; pedisulcus (Figure 1D) well developed; claw (Figure 1E) with large basal tooth 0.53 times length of claw. ***Wing***. Length 1.6 mm. Costa with dark spinules and hairs except basal patch of yellow hairs. Subcosta with dark hairs except near apex. Base of radius with tuft of yellow hairs. Basal portion of radius fully haired. R_1 with dark spinules and hairs; R_2 with hairs only. Basal cell absent. ***Halter***. White except basal portion darkened. ***Abdomen***. Basal scale ochreous, with fringe of whitish yellow hairs. Dorsal surface of abdomen medium to dark brown except segment 2 light brown, moderately covered with dark short to long hairs; tergites of segments 2 and 6–8 shiny when illuminated at certain angles. Sternal plate on segment 7 undeveloped. ***Terminalia***. Sternite 8 (Figure 1F) bare medially, with 17 or 18 medium-long to long hairs together with four slender short hairs on each side. Ovipositor valves (Figure 1F) triangular (although posteromedial corner rounded), thin, membranous, each moderately covered with microsetae interspersed with two or three short hairs; inner margins gently concave, somewhat sclerotized, and moderately separated from each other. Genital fork (Figure 1G) of usual inverted-Y form, with slender stem; arms of moderate width, strongly folded medially. Paraproct in ventral view (Figure 1H) shallowly concave along anteromedial margin, with three sensilla on anteromedial surface; paraproct in lateral view (Figure 1I) somewhat produced ventrally beyond ventral tip of cercus, 0.68 times as long as wide, with 22 or 23 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Figure 1I) short, rounded posteriorly, 0.50 times as long as wide. Spermatheca (Figure 1J) ellipsoidal, 1.5 times as long as its greatest width, well sclerotized and darkened except duct and small area near juncture with duct unpigmented, and with many fissures on outer surface; internal setae absent; both accessory ducts unpigmented, slender, slightly thicker in diameter than major one.

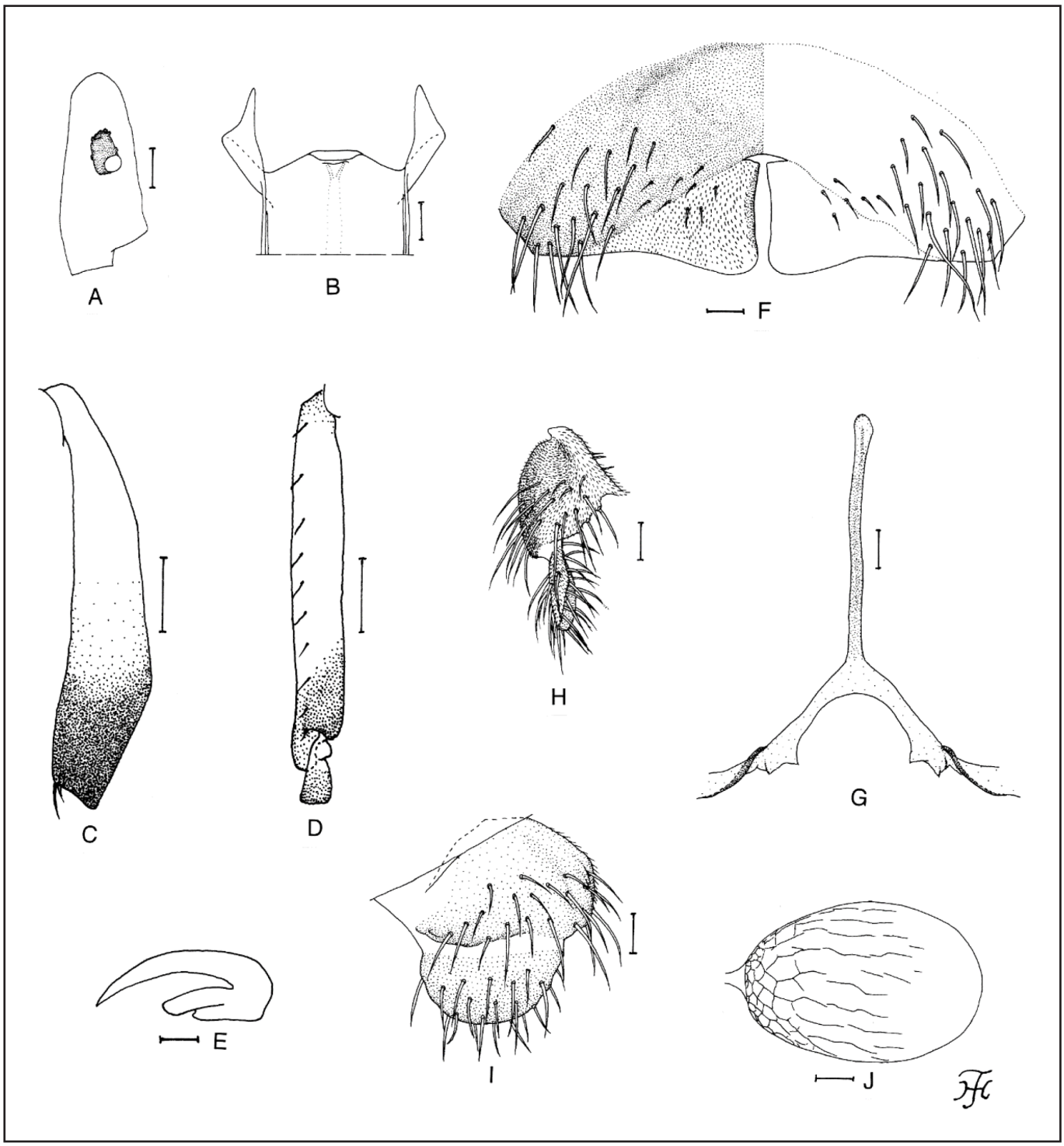

Figure 1. Female of *S. ogatai*. A, sensory vesicle (right side; front view). B, cibarium (front view). C, hind tibia (left side; outer view). D, hind basitarsus and second tarsomere (left side; outer view). E, claw. F, sternite 8 and ovipositor valves (ventral view). G, genital fork (ventral view). H & I, paraprocts and cerci (right side; H, ventral view; I, lateral view). J, spermatheca. Scale bars. 0.1 mm for C and D; 0.02 mm for A, B and F–J; 0.01 mm for E.

Male (n=1). Body length 2.0 mm. ***Head***. Slightly wider than thorax. Upper eye medium brown, consisting of large facets in 15 vertical columns and 16 horizontal rows on each side. Clypeus brownish black, whitish pruinose, densely covered with whitish yellow scale-like short hairs (mostly directed upward) interspersed with several dark brown longer hairs near lower margin on each side. Antenna composed of scape, pedicel and nine flagellomeres, light to medium brown except scape and pedicel and base of first flagellomere whitish yellow; first flagellomere elongate, 1.46 times length of second. Maxillary palpus light to medium brown, with five segments, proportional lengths of third, fourth, and fifth segments 1.00:1.29:3.02; third segment (Figure 2A) slender, widened apically; sensory vesicle (Figure 2A) small, globular or ellipsoidal (0.10–0.14 times length of third segment), and with small opening. ***Thorax***. Scutum brownish black except anterolateral calli dark brown, shiny widely along both lateral margins and on prescutellar area when illuminated at certain angles, and densely covered with whitish-yellow scale-like recumbent short hairs. Scutellum dark brown, covered with yellow short hairs and dark brown long upright hairs along posterior margin. Postnotum brownish black, slightly shiny when illuminated at certain angles, and bare. Pleural membrane ochreous and bare. Katepisternum dark brown, longer than deep, shiny when illuminated at certain angles, moderately covered with yellow and brown fine short hairs. ***Legs***. Color nearly as in female except fore and mid trochanter light brown, fore tibia yellowish white except little less than apical one-third dark brown, hind tibia medium to dark brown except basal two-fifths yellowish white, hind basitarsus dark brown except basal half yellowish white, and hind second tarsomere dark brown except basal one-third yellow. Fore basitarsus slightly dilated, 7.19 times as long as its greatest width. Hind basitarsus (Figure 2B) spindle-shaped, enlarged, 3.79 times as long as wide, and 0.83 and 1.00 times as wide as greatest width of hind tibia and femur, respectively; calcipala (Figure 2B) nearly as long as basal width, and 0.34 times as wide as greatest width of basitarsus; pedisulcus (Figure 2B) well developed. ***Wing***. Length 1.7 mm. Other characteristics as in female except subcosta bare. ***Halter***. Yellowish grey except basal stem darkened. ***Abdomen***. Basal scale dark brown, with fringe of light to dark brown hairs. Dorsal surface of abdomen medium brown to brownish black, covered with dark brown short to long hairs except parts of segment 2 with yellowish hairs; segments 2 and 5–7 each with pair of shiny dorsolateral or lateral patches. ***Genitalia***. Coxite in ventral view (Figure 2C) nearly rectangular, 2.1 times as long as its greatest width. Style in ventral view (Figure 2C) bent inward, with single apical spine; style in ventrolateral view (Figure 2D) somewhat tapered from base to apical one-third, then nearly parallel-sided to round apex, 2.55 times as long as its greatest width at base, and 0.8 times length of coxite. Ventral plate in ventral view (Figure 2C) with body transverse, 0.51 times as long as wide, narrowed posteriorly, with anterior margin produced anteromedially, posterior margin somewhat concave ventromedially, and densely covered with microsetae on ventral surface; basal arms of moderate length, nearly parallel-sided; ventral plate in lateral view (Figure 2E) moderately produced ventrally; ventral plate in caudal view (Figure 2F) with ventral margin nearly straight, densely covered with microsetae on posterior surface. Median sclerite arising near anterior tip of ventral plate (Figure 2E) and broad, plate-like in caudal view (Figure 2G). Parameres (Figure 2H) of moderate size, each with four stout hooks decreasing in length from base to apex. Aedeagal membrane (Figure 2H) moderately covered with microsetae; dorsal plate not defined. Ventral surface of abdominal segment 10 (Figure 2I, J) moderately sclerotized near anterior margin and without distinct hairs near posterolateral corners. Cercus (Figure 2I, J) small, with 13 or 14 hairs.

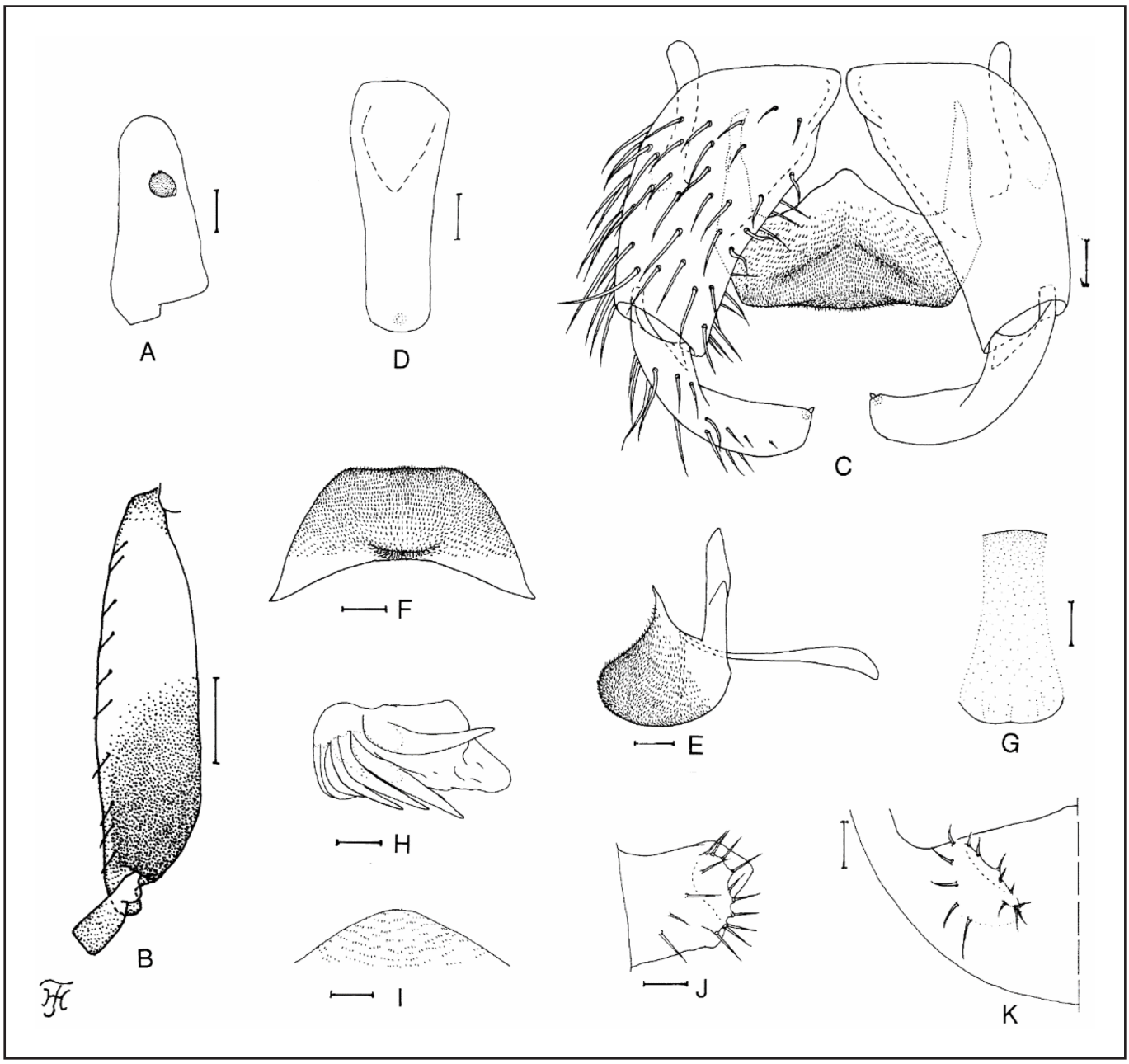

Figure 2. Male of *S. ogatai*. A, sensory vesicle (right side; front view). B, hind basitarsus and second tarsomere (left side; outer view). C, coxites, styles and ventral plate (ventral view). D, style (right side; ventrolateral view). E, ventral plate and median sclerite (lateral view). F, ventral plate (caudal view). G, median sclerite (ventral view). H, paramere (left side; caudal view). I, aedeagal membrane (caudal view). J and K, 10th abdominal segments and cerci (right side; J, lateral view; K, caudal view). Scale bars. 0.1 mm for B; 0.02 mm for A and C–K.

Pupa (n=2). Body length 2.4 mm. ***Head***. Integument ochreous, moderately covered with small round tubercles except antennal sheaths and ventral surface almost bare; frons with three pairs of unbranched long trichomes with or without coiled apices (Figure 3A); face with pair of unbranched long trichomes with coiled apices (Figure 3B); three frontal trichomes on each side arising close together, subequal in length to one another and longer than facial one. ***Thorax***. Integument yellow to ochreous, moderately covered with round tubercles, and with three long dorsomedial trichomes with coiled apices (gradually shortened posteriorly (Figure 3C), two long anterolateral trichomes (anterior trichome more slender with straight apex, posterior one with coiled apex) (Figure 3D), one medium-long mediolateral trichome with straight apex (Figure 3E), and three ventrolateral trichomes (two medium-long, one extremely short) with straight apices (Figure 3F), on each side; all trichomes unbranched. Gill (Figure 3G) composed of eight slender thread-like filaments, arranged as [(1+2)+(1+2)]+2 or [(2+1)+(1+2)]+2 from dorsal to ventral, with medium-long common basal stalk having somewhat swollen transparent basal fenestra; common basal stalk 0.71–0.80 times length of interspiracular trunk; dorsal and middle triplets sharing medium-long stalk, which is 0.78–0.90 times as long as common basal stalk; stalk of ventral pair of filaments medium-long, 1.36–1.60 times length of common basal stalk, and 1.13–1.23 times length of interspiracular trunk; dorsal triplet with short primary and secondary stalks, middle triplet with medium-long primary and short to medium-long secondary stalks; primary stalk of dorsal triplet lying against that of ventral pair at angle of 60–70 degrees when viewed laterally; filaments of dorsal triplets 1.6–1.7 mm long, those of middle triplets 1.9 mm long, and those of ventral pair 2.2 mm long; two filaments of ventral pair subequal in thickness to each other and 1.5 and 1.3 times as thick as filaments of dorsal and middle triplets, respectively, when compared basally; all filaments light brown, gradually tapered toward apex; cuticle of all filaments with well-defined annular ridges and furrows though becoming less marked apically, densely covered with microtubercles. ***Abdomen***. Dorsally, all segments light to medium yellow except segments 1, 2 and 9 ocherous, and without microtubercles; segment 1 with one unbranched slender medium-long hair-like seta (Figure 3H) on each side; segment 2 with one unbranched slender medium-long hair-like seta and five minute setae submedially (Figure 3I) on each side; segments 3 and 4 each with four hooked spines and one minute seta on each side; segment 5 lacking spine-combs and comb-like groups of minute spines on each side; segments 6–9 each with spine-combs (those on segment 9 somewhat smaller than those on segments 6–8) in transverse row and comb-like groups of micro-spines on each side; segment 5 with four minute setae on each side; segments 6–8 each with two minute setae on each side; segment 9 with pair of triangular terminal hooks (Figure 3J). Ventrally, segment 4 with two unbranched hooks (Figure 3K) (shorter than those on segments 5–7) and few slender short setae, on each side; segment 5 with pair of bifid hooks (Figure 3L) submedially and few short slender setae on each side; segments 6 and 7 each with pair of bifid inner and unbranched outer hooks somewhat spaced from each other and few short slender setae on each side; segments 4–8 each with comb-like groups of micro-spines. Each side of segment 9 with three grapnel-shaped hooklets. ***Cocoon*** (Figure 3M). Wall-pocket-shaped, thinly woven, moderately extended ventrolaterally; anterior margin with anterodorsal projection; posterior half with floor roughly woven; individual threads invisible; 3.0 mm long by 2.0 mm wide.

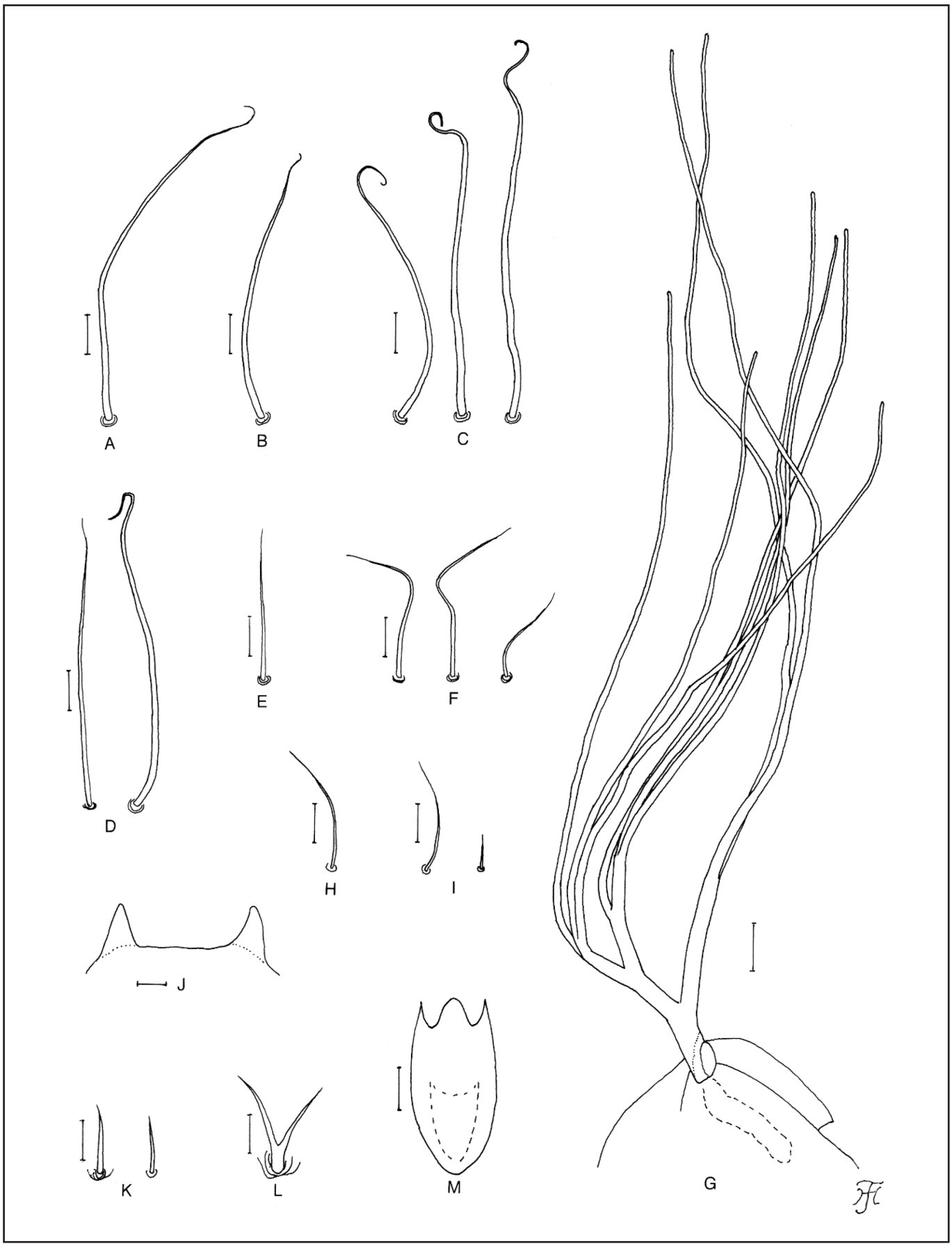

Figure 3. Pupa of *S. ogatai.* A, frontal trichome. B, facial trichome. C–F, thoracic trichomes (C, mediodorsal; D, anterolateral; E, mediolateral; F, ventrolateral). G, gill filaments (right side; outer view). H, hair-like seta on dorsum of abdominal segment 1. I, hair-like seta and minute seta on dorsum of abdominal segment 2. J, terminal hooks (caudal view). K, unbranched hooklets on ventral surface of abdominal segment 4. L, bifid hooklet on ventral surface of abdominal segment 5. M, cocoon (dorsal view). Scale bars. 1.0 mm for M; 0.1 mm for G; 0.02 mm for A–F, H, I, K and L; 0.01 mm for J.

Remarks. Morphological characteristics of the female of *S. ogatai* are described and illustrated (Figure 1A–J) for the first time. In addition, information on the number of upper-eye (large) facets, size of the sensory vesicle (Figure 2A), color of the legs, and relative length of the fore and hind basitarsi (Figure 2B) in the male, and the length of trichomes on the frons and thorax (Figure 3A–F), and the shape of the terminal hooks (Figure 3J) in the pupa is also presented.

This species is characterized in the female and male by the base of the radius with a tuft of yellow hairs; in the female by the head slightly wider than the thorax and 5.89 times as wide as the greatest width of the frons; in the male by upper-eye (large) facets in 15 vertical columns and 16 horizontal rows, and hind basitarsus spindle-shaped, enlarged, 3.79 times as long as wide, and 0.83 and 1.00 times as wide as the greatest width of the hind tibia and femur, respectively; and in the pupa by the terminal hooks simple and cone-like, and the cocoon with an anterodorsal projection. These characteristics combined separate *S. ogatai* from the other species in the *S. ceylonicum* species-group.

The placement of *S. ogatai* in the *S. ceylonicum* species-group is confirmed by the shape of the male ventral plate, which is narrowed posteriorly when viewed ventrally (Figure 2C), agreeing with the diagnosis of this species-group (Takaoka, 2012). Furthermore, the pupal terminal hooks, which are simple and cone-like (Figure 3J), and the larval postgenal cleft, which is long, 5.5 times as long as the postgenal bridge (Orii *et al*., 1969), are similar to those of species in this species-group (Takaoka, 2012).

This species is exceptional among species in the S. *ceylonicum* species-group by having the yellow tuft hairs on the base of the radius in both the female and male, and short female sensory vesicle, which are, though, shared by *S. longitruncum* Takaoka & Davies from Peninsular Malaysia (Takaoka & Davies, 1995). However, *S. ogatai* is easily distinguished from *S. longitruncum* in the female by the relative width of the head against the greatest width of the frons (5.89 in this species versus 4.5 in *S. longitruncum*), in the male by the number of upper-eye (large) facets (15 vertical columns and 16 horizontal rows in this species versus 13 vertical columns and 14 horizontal rows in *S. longitruncum*), and in the pupa by the length of the common basal stalk of the gill (medium-long in this species versus long in *S. longitruncum*), and cocoon with an anterodorsal projection (without an anterodorsal projection in *S. longitruncum*).

The female of *S. ogatai* is similar to that of *S. atratoides* Takaoka & Davies from Java, Indonesia (Takaoka & Davies, 1996), by having the relatively wider head against the greatest width of the frons and yellow tuft hairs on the base of the radial vein, but is distinguished from the latter species by the mandible with outer teeth (without outer teeth in *S. atratoides*), sensory vesicle short (elongate, 0.66 times as long as the third palpal segment in *S. atratoides*), and cibarium with a sclerotized plate folded forward from the posterior margin (without such a plate in *S. atratoides*).

This species is clearly distinguished from two other related species, *S. okinawense* and *S. tokarense* from the Nansei Islands (Takaoka, 1973, 1976), in the female by the relative width of the head against the greatest width of the frons, which is 5.89 (4.09–4.70 in the latter two species); in the male by the hind basitarsus 0.83 times as wide as the hind tibia (as wide as or slightly wider than the hind tibia in the latter two species); in the pupa by the terminal hooks simple and cone-like (wide, plate-like in the latter two species); and in the larva by the postgenal cleft much longer than the postgenal bridge (medium-long, nearly as long as or somewhat longer than the postgenal bridge in the latter two species).

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Declaration of Competing Interest

We declare that this is our original work. It has not been published elsewhere and we have no conflicts of interest concerning the work reported in this paper. All authors have contributed to this study throughout the study design, field work, data collection, data analyses and data interpretation. The authors have read and approved the manuscript.

REFERENCES

Adler, P.H. (2021). World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory [2021]. https://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf. Accessed 1 February 2022.

Adler, P.H., Currie, D.C. & Wood, D.M. (2004). The Black Flies (Simuliidae) of North America. USA, New York, Ithaca: Cornell University Press.

Bentinck, W. (1955). The black flies of Japan and Korea (Diptera: Simuliidae). Japan, Tokyo, US Department of the Army: Contributions of the 406th Medical General Laboratory, pp. 23.

Fukuda, M., Choochote, W., Bain, O., Aoki, C. & Takaoka, H. (2003). Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Japanese Journal of Tropical Medicine and Hygiene* **31**: 99-102. https://doi.org/10.2149/tmh1973.31.99

Orii, T., Uemoto, K. & Onishi, O. (1969). Key to larval stage of black flies (Simuliidae) of Japan. *Sanitary Injurious Insects* **13**: 1-13.

Pramual, P., Tangkawanit, U., Kunprom, C., Vaisusuk, K., Chatan, W., Wongpakam, K. & Thongboonma, S. (2020). Seasonal population dynamics and a role as natural vector of *Leucocytozoon* of black fly, *Simulium chumpornense* Takaoka & Kuvangkadilok. *Acta Tropica* **211**: 105617. https://doi.org/10.1016/j.actatropica.2020.105617

Rubtsov, I.A. (1962). Simuliidae (Melusinidae) (part). *Die Fliegen der Palaearktischen Region (III)* **14**: 305-33.

Takaoka, H. (1973). Descriptions of 2 new species of blackflies, *Simulium* (*Gomphostilbia*) *tokarense* and *S*. (*Eusimulium*) *morisonoi* (Diptera: Simuliidae), from the Tokara Islands, Japan. *Japanese Journal of Sanitary Zoology* **23**: 201-207.

Takaoka, H. (1976). Studies on blackflies of the Nansei Islands, Japan (Simuliidae; Diptera): II. On six species of the subgenera, *Gomphostilbia* Enderlein, *Morops* Enderlein, *Odagmia* Enderlein and *Gnus* Rubzov, with the description of *Simulium* (*Gomphostilbia*) *okinawense* sp. nov. *Japanese Journal of Sanitary Zoology* **27**: 385-398. https://doi.org/10.7601/mex.27.385

Takaoka, H. (2003). The Black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Japan, Fukuoka: Kyushu University Press, pp. 581.

Takaoka, H. (2012). Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in the Oriental Region. *Zootaxa* **3577**: 1-42. https://doi.org/10.11646/zootaxa.3577.1.1

Takaoka, H. & Davies, D.M. (1995). The Black Flies (Diptera: Simuliidae) of West Malaysia. Japan, Fukuoka: Kyushu University Press, pp. 175.

Takaoka, H. & Davies, D.M. (1996). The Black Flies (Diptera: Simuliidae) of Java, Indonesia. *Bishop Museum Bulletin in Entomology* **6**: 88.

Thaijarern, J., Tangkawanit, U., Wongpakam, K. & Pramual, P. (2019). Molecular detection of *Trypanosoma* (Kinetoplastida: Trypanosomatidae) in black flies (Diptera: Simuliidae) from Thailand. *Acta Tropica* **200**: 105196. https://doi.org/10.1016/j.actatropica.2019.105196